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DEVELOPMENT AND VALIDATION OF AN ANALYTICAL METHOD FOR STIMULATION OF RIVASTIGMINE AND DONEPEZIL IN BULK AND PHARMACEUTICAL DOSAGE FORM BY USING UPLC

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ABSTRACT

A simple, Accurate, precise method was developed for the simultaneous estimation of the Donepezil and Rivastigmine in bulk and pharmaceutical dosage form. Chromatogram was run through CHS (100mm * 2.1mm, 1.7µ). Mobile phase containing 0.01N Kh2po4 Buffer and Acetonitrile in the ratio of 70:30 v/v was pumped through column at a flow rate of 0.3 ml/min. Temperature was maintained at 30°C. Optimized wavelength for Donepezil and Rivastigmine was 238nm. Retention time of Donepezil and Rivastigmine were found to be 1.027 min and 1.526 min. %RSD of the Donepezil and Rivastigmine were and found to be 0.8 and 0.9 respectively. %Recovery was Obtained as 99.18% and 99.48% for Donepezil and Rivastigmine. LOD, LOQ values were obtained from regression equations of Donepezil and Rivastigmine were 0.09ppm, 0.28 ppm and 0.03ppm, 0.08ppm respectively. Regression equation of Donepezil is y = 80824x + 10133, and of Rivastigmine is y = 87497x + 2312.6. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

INTRODUCTION:

Rivastigmine:

Rivastigmine is a cholinesterase inhibitor used to treat mild to moderate dementia in Alzheimer's and Parkinson's. Rivastigmine is a carbamate derivative that is structurally related to physostigmine, but not to donepezil and tacrine. The precise mechanism of rivastigmine has not been fully determined, but rivastigmine binds reversibly with and inactivates cholinesterase (examples: acetylcholinesterase, butyrylcholinesterase), preventing hydrolysis of acetylcholine and thus leading to an increased concentration of acetylcholine at cholinergic synapses. The anticholinesterase activity of rivastigmine is relatively specific for brain acetylcholinesterase and butyrylcholinesterase compared with those in peripheral tissues.

Donepezil:

The main pharmacological actions of this drug are believed to occur as the result of this enzyme inhibition, enhancing cholinergic transmission, which relieves the symptoms of Alzheimer's dementia. In addition to the above, other mechanisms of action of donepezil are possible, including the opposition of glutamate-induced excitatory

transmission via downregulation of NMDA receptors and the regulation of amyloid proteins, which have demonstrated significant effects on the disease process of Alzheimer's.8,12,17 Other possible targets for donepezil may also include the inhibition various inflammatory signaling pathways, exerting neuroprotective effects

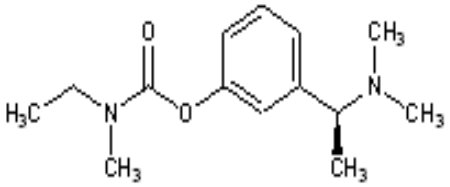

Fig:1-Structure of Rivastigmine

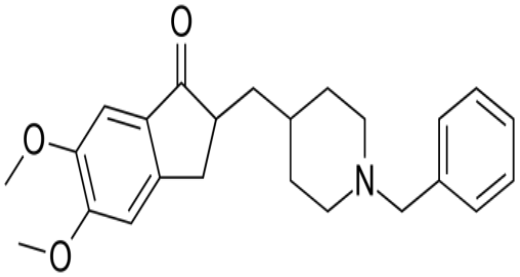

Fig:2-Structure of Donezepil

MATERIALS AND METHODS

Donepezil and Rivastigmine, Combination of Donepezil and Rivastigmine (Synthetic mixture) dosage forms, distilled water, acetonitrile, phosphate buffer, ammonium acetate buffer, glacial acitic acid, methanol, potassium dihydrogen phosphate buffer, tetra hydrofuran, tri ethyl amine, ortho-phosphoric acid etc.

Instrument:

- Electronics Balance-Denver
- pH meter -BVK enterprises, India
- Ultrasonicator-BVK enterprises
- UPLC - WATERS ACQUITY UPLC SYSTEM with Auto Injector and TUV (Tunable UV) Detector.
- Lab India UV double beam spectrophotometer with UV win5 software was used for measuring absorbances of Donepezil and Rivastigmine solutions.

Method Development:

Preparation of buffer:

0.01N Kh2po4 Buffer: Accurately weighed 1.36gm of sodium hydrogen phosphate in a 1000ml of Volumetric flask add about 900ml of milli-Q water added and degas to sonicate and finally make up the volume with water to get 0.01N Kh2po4 buffer.

Diluent: Based up on the solubility of the drugs, diluent was selected, Acetonitrile and Water taken in the ratio of 50:50

Preparation of Standard stock solutions: Accurately weighed 10mg of Donepezil, 1.5mg of Rivastigmine and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Donepezil and 30µg/ml Rivastigmine)

Preparation of Standard working solutions (100% solution): 1ml from each stock solution was pipetted out and taken into a 10ml volumetric flask and made up with diluent. (20µg/ml of Donepezil and 3µg/ml of Rivastigmine).

Sample Preparation:

Synthetic mixture wa prepared and then Weighed equivalent to 1 Capsule was transferred into a 100mL volumetric flask, 50mL of diluent added and sonicated for 25 min, further the volume made up with diluent and filtered. (100µg/ml of Donepezil and 15µg/ml Rivastigmine)

From the filtered solution 2 ml was pipeted out into a 10 ml volumetric flask and made upto 10ml with diluent. (20µg/ml of Donepezil and 3µg/ml of Rivastigmine).

Many trials has carried during development and it has been done by changing columns and Mobile phases, those were reported below.

Method Validation:

System suitability:

The system suitability parameters like theoretical plates, tailing factor, resolution were evaluated from standard chromatograms. The standard and sample solutions were injected five times and peak areas of injections were measured in HPLC. The % RSD for the area of five replicate injections was found to be within the specified limits.

Three replicate injections of standard and sample solutions were injected and the assay was calculated by using the formula:

$$\%\ ASSAY = \frac{\text{Sample area}}{\text{Standard area}} \times \frac{\text{Weight of standard}}{\text{Dilution of standard}} \times \frac{\text{Dilution of sample}}{\text{Weight of sample}} \times \frac{\text{Purity}}{100} \times \frac{\text{Weight of tablet}}{\text{Label claim}} \times 100$$

Linearity:

Preparation of Standard stock solutions: Accurately weighed 10mg of Donepezil, 1.5mg of Rivastigmine and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Donepezil and 30µg/ml Rivastigmine)

25% Standard solution: 0.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (5µg/ml of Donepezil and 0.75µg/ml of Rivastigmine)

50% Standard solution: 0.5ml each from two standard stock solutions was pipetted out and made up to 10ml. (10µg/ml of Donepezil and 1.5µg/ml of Rivastigmine)

75% Standard solution: 0.75ml each from two standard stock solutions was pipetted out and made up to 10ml. (15µg/ml of Donepezil and 2.25µg/ml of Rivastigmine)

100% Standard solution: 1.0ml each from two standard stock solutions was pipetted out and made up to 10ml. (20µg/ml of Donepezil and 3µg/ml of Rivastigmine)

125% Standard solution: 1.25ml each from two standard stock solutions was pipetted out and made up to 10ml. (25µg/ml of Donepezil and 3.75µg/ml of Rivastigmine)

150% Standard solution: 1.5ml each from two standard stock solutions was pipetted out and made up to 10ml (30µg/ml of Donepezil and 4.5µg/ml of Rivastigmine)

Accuracy:

Preparation of Standard stock solutions: Accurately weighed 10mg of Donepezil, 1.5mg of Rivastigmine and transferred to 50ml volumetric flask. 3/4 th of diluents was added to the flask and sonicated for 10 minutes. Flask was made up with diluents and labeled as Standard stock solution. (200µg/ml of Donepezil and 30µg/ml Rivastigmine)

Preparation of 50% Spiked Solution: 0.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 100% Spiked Solution: 1.0ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Preparation of 150% Spiked Solution: 1.5ml of sample stock solution was taken into a 10ml volumetric flask, to that 1.0ml from each standard stock solution was pipetted out, and made up to the mark with diluent.

Acceptance Criteria:

The % Recovery for each level should be between 98.0 to 102

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Robustness conditions like Flow minus (0.9ml/min), Flow plus (1.1ml/min), mobile phase minus, mobile phase plus, temperature minus (25°C) and temperature plus(35°C) was maintained and samples were injected in duplicate manner. System suitability parameters were not much affected and all the parameters were passed. %RSD was within the limit.

LOD sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flasks and made up with diluents. From the above solutions 0.1ml each of Rivastigmine, Donepezil, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluents

LOQ sample Preparation: 0.25ml each from two standard stock solutions was pipetted out and transferred to two separate 10ml volumetric flask and made up with diluent. From the above solutions 0.3ml each of Rivastigmine, Donepezil, solutions respectively were transferred to 10ml volumetric flasks and made up with the same diluent.

Results and Discussion :

Optimized Chromatographic conditions:

Mobile phase **:** Acetonitriile: 0.01N Kh2po4 (65:35 v/v)

Flow rate **:** 0.3ml/min

Column **:** CHS C18 (2.1 x 100mm, 1.7µm)

Detector wave length **:** 238nm

Column temperature **:** 30°C

Injection volume **:** 2µL

Run time **:** 10 min

Diluent **:** Water and Acetonitrile in the ratio 50:50

Results : In this trail Both peaks have good resolution, tailing Factor, theoretical plate count and resolution.

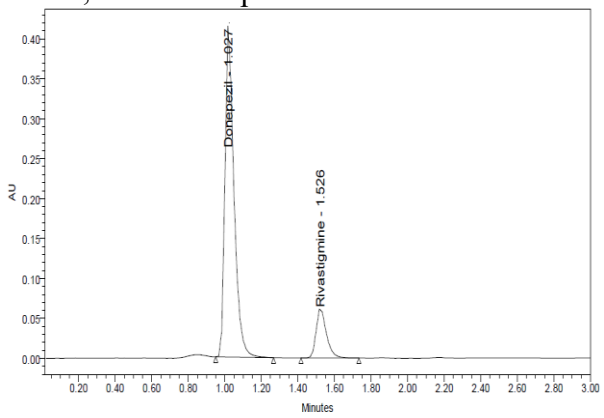

Fig.3: Optimized chromatogram of Donepezil and Rivastigmine

Observation: Donepezil and Rivastigmine were eluted at 1.027 min and 1.526 min respectively with good resolution. Plate count and tailing factor was very satisfactory, so this method was optimized and to be validated.

Method Validation:

System suitability: All the system suitability parameters are within range and satisfactory as per ICH guidelines.

Assay: Standard preparations are made from the API and Sample Preparations are from Formulation. Both sample and standards are injected six homogeneous samples. Drug in the formulation was estimated by taking the standard as the reference. The Average %Assay was calculated and found to be 100.11% and 99.94% for Donepezil and Rivastigmine respectively.

Linearity: Six Linear concentrations of Donepezil (5ppm-30ppm) and Rivastigmine (0.75ppm to 4.5ppm) are prepared and injected. Regression equation of the the Donepezil and Rivastigmine are found to be, y = 80824x + 10133 and y = 87497x + 2312.6 and the regression co-efficient was 0.999.

Accuracy: Three concentrations 50%, 100%, 150%, were injected in a triplicate manner and amount Recovered and % Recovery were displayed in Table 6.5.

Precision: Repeatability precision was performed and % RSD for Donepezil and Rivastigmine were found to be 0.8% and 0.9% respectively.

Robustness: Small deliberate changes in method like Flow rate, mobile phase ratio, and temperature are made but there were no recognized change in the result and are within range as per ICH Guide lines.

Table-1: System suitability studies of Donepezil and Rivastigmine method

S no	Donepezil			Rivastigmine			
Inj	RT(min)	USP Plate Count	Tailing	RT(min)	USP Plate Count	Tailing	Resoluton
1	1.019	2827	1.38	1.521	4151	1.28	5.1
2	1.019	2844	1.39	1.522	4183	1.29	5.1
3	1.019	2863	1.38	1.524	4252	1.30	5.1
4	1.031	2776	1.37	1.535	4315	1.29	5.3
5	1.031	2803	1.39	1.535	4246	1.29	5.2
6	1.031	2813	1.38	1.535	4281	1.28	5.3

Acceptance Criteria: Theoretical Plates: > 2000

Tailing factor: <2

Observed Value: < 2

Table-2: Assay of Drug

S. No.	Donepezil %Assay	Rivastigmine %Assay
1	98.93	99.26
2	99.99	100.83
3	101.11	100.03
4	99.50	98.79
5	100.89	100.08
6	100.26	100.63
AVG	100.11	99.94
STDEV	0.83	0.784
%RSD	0.83	0.8

Acceptance Criteria: 98-102
Observed Value: Donepezil – 100.11%
Rivastigmine – 99.94%

Table-3: Calibration data of Donepezil and Rivastigmine method.

S.no	Concentration Donepezil (µg/ml)	Response	Concentration Rivastigmine (µg/ml)	Response
1	0	0	0	0
2	5	410334	0.75	66264
3	10	825131	1.5	137718
4	15	1237271	2.25	197799
5	20	1631875	3	270065
6	25	2029324	3.75	329653
7	30	2423568	4.5	392773

Acceptance Criteria: $R^2 = 0.999$
Observed Value: $R^2 = 0.999$

Table-4: Accuracy results of Donepezil

% Level	Amount Spiked µg/mL	Amount recovered µg/mL	% Recovery	Mean %Recovery
50%	10	9.903494	99.03	99.18%
	10	9.819014	98.19	
	10	9.955743	99.56	
100%	20	19.82938	99.15	
	20	19.77862	98.89	
	20	20.08579	100.43	
150%	30	29.72743	99.09	
	30	29.69129	98.97	
	30	29.80039	99.33	

Table-5: Accuracy results of Rivastigmine

% Level	Amount Spiked µg/mL	Amount recovered µg/mL	% Recovery	Mean %Recovery
50%	1.5	1.482433	98.83	99.48%
	1.5	1.495096	99.67	
	1.5	1.497782	99.85	
100%	3	2.970221	99.01	
	3	3.013696	100.46	
	3	2.988427	99.61	
150%	4.5	4.478844	99.53	
	4.5	4.469529	99.32	
	4.5	4.455346	99.01	

Acceptance Criteria: % Recovery: 98-102%
Observed Value: Donepezil – 99.18%
Rivastigmine – 99.48%

Table-6: System precision results for Donepezil and Rivastigmine

S.No.	Donepezil	Rivastigmine
1	1607550	267418
2	1638110	261318
3	1614930	264602
4	1624202	266814
5	1642932	266513
6	1624705	267640
Mean	1625405	265718
Std. Dev.	13409.5	2409.3
%RSD	0.8	0.9

Acceptance Criteria: % RSD: < 2
Observed Value: Donepezil – 0.8
Rivastigmine – 0.9

Table-7: Robustness data of Donepezil and Rivastigmine method.

S.NO	Robustness condition	Donepezil %RSD	Rivastigmine %RSD
1	Flow minus(0.27ml/min)	0.7	0.4
2	Flow Plus(0.33ml/min)	0.4	1.0
3	Mobile phase minus(75:25% v/v)	0.2	0.5
4	Mobile phase Plus(65:35% v/v)	0.7	0.8
5	Temperature minus(27^{O}C)	0.5	0.6
6	Temperature Plus(33^{O}C)	0.5	0.7

CONCLUSION

A simple, Accurate, precise method was developed for the simultaneous estimation of the Donepezil and Rivastigmine in bulk and pharmaceutical dosage form. Retention time of Donepezil and Rivastigmine were found to be 1.027 min and 1.526 min. %RSD of the Donepezil and Rivastigmine were and found to be 0.8 and 0.9 respectively. %Recovery was Obtained as 99.18% and 99.48% for Donepezil and Rivastigmine. LOD, LOQ values were obtained from regression equations of Donepezil and Rivastigmine were 0.09ppm, 0.28 ppm and 0.03ppm, 0.08ppm respectively. Regression equation of Donepezil is y = 80824x + 10133, and of Rivastigmine is y = 87497x + 2312.6. Retention times are decreased and that run time was decreased so the method developed was simple and economical that can be adopted in regular Quality control test in Industries.

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